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Antioxidant and antipyretic studies on *Pothos scandens* L

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ABSTRACT

Objective: To investigate *Pothos scandens* for the *in vitro* antioxidant and antipyretic activity. **Methods:** Preliminary phytochemicals, total phenolics and flavonoid contents were analyzed in leaf, stem and root samples. *In vitro* antioxidant activity was evaluated by different assays such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2, 2′-azinobis -3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) radical scavenging, ferric-reducing antioxidant power (FRAP) assay, phosphomolybdenum reduction assay, metal chelating activity, superoxide anion radical scavenging activity, hydrogen peroxide and nitric oxide scavenging assay. The antipyretic activity of root methanol extract was studied by pyrexia induced by brewer′s yeast on Wistar albino rats at concentration of 200 and 400 mg/kg using paracetamol as standard drug. **Results:** The total phenolics and tannin content were found to be higher in ethanol extract of stem, whereas total flavonoid content was higher in acetone extract of root. The methanol extract of root showed highest free radical scavenging activity in assays namely ABTS assay (8 221.5 μM TE/g extract), FRAP assay [514.4 mM Fe (Ⅱ)/g extract], hydrogen peroxide (60.3%) and nitric oxide scavenging assays (58.7%). The DPPH assay and superoxide radical assay results revealed that the ethanol extract of root has remarkable free radical scavenging capacity (IC_{50} 0.284 mg/mL and 70.84%). The antipyretic studies on methanol extract of root showed significant reduction of temperature in pyrexia induced rats at 200 and 400 mg/kg doses. **Conclusions:** These findings justify that *Pothos scandens* can be a valuable natural antioxidant and antipyretic source which seemed to provide potential nutraceuticals for human health.

1. Introduction

Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and communities in many developing countries[1]. For thousands of years, these natural plant products have been utilized for human healthcare in the form of drugs, antioxidants, flavours, fragrances, dyes, insecticides and pheromones. However, during the last century the use of synthetic drugs led to a decline in the use of plant-derived compounds, so that the synthetic drugs would perhaps completely replace the use of traditional plant-derived medicines[2].

Naturally, there is a dynamic balance between the amount of free-radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects[3]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body′s normal use of oxygen such as respiration and some cell-mediated immune functions. They are also found or generated through environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants, pesticides *etc*. However, the amount of these protective antioxidant principles present under the normal physiological conditions, are sufficient only to cope with the physiological rate of free-radical generation. Therefore, it is obvious that any additional burden of free-radicals either from environment or produced within the body can alter the pro-oxidant and antioxidant balance leading to oxidative stress.

Reactive oxygen species (ROS) is a collective term for oxygen-centered radicals such as superoxide, hydroxyl and non- radical oxygen derivatives, namely hydrogen peroxide and singlet oxygen[4]. In humans the over production of ROS can result in tissue injury and has been implicated in disease progression and oxidative damage of nucleic acids and proteins[5]. When there is a lack of

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antioxidants to quench the excess reactive free radicals, cardiovascular, cancer, neurodegenerative, Alzheimer's and inflammatory diseases may develop in the body[6]. Thus the antioxidant status in human reflects the dynamic balance between the antioxidant defense and pro-oxidant conditions and has been suggested as a useful tool in estimating the risk of oxidative damage[3]. Due to the benefits of antioxidants, food and pharmaceutical products are normally supplemented with synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *tert*-butyl hydroxyquinine (TBHQ). However, the dietary intake of synthetic antioxidants could cause genotoxicity and carcinogenicity at high concentrations[7]. In addition, natural antioxidants from plant products may be more effective in reducing ROS levels compared to synthetic single dietary antioxidants due to the synergistic actions of a wide range of biomolecules such as vitamins C and E, phenolic compounds, carotenoids, terpenoids and phytomicronutrients[8].

Antipyretics are drugs that reduce body temperature in situations such as fever which is a part of the body's immune response to infection. However, they will not affect the normal body temperature if one does not have a fever. A rise in blood temperature leads to manifestations of heat loss which increases heat production. Antipyretics cause the hypothalamus to override an interleukin-induced increase in temperature. The body will then work to lower the temperature and the result is a reduction in fever. The non-steroidal anti-inflammatory drugs are antipyretic, anti-inflammatory, and pain relievers[9]. Search for herbal remedies with potent antipyretic activity received momentum recently as the available antipyretics, such as paracetamol, nimusulide *etc.* have toxic effect to the various organs of the body[10].

Pothos scandens (*P. scandens*) is a medicinal aroid, which belongs to the family Araceae. The bruised root of the plant is applied to promote healing of abscesses, after being fried in oil. The Indian people use an infusion of the leaves of this plant as a bath for curing convulsions and epilepsy. Apart from that, the stem is also widely used to treat asthma, after being cut up with camphor and smoked like tobacco. It has been also reported that the whole plant is used against various health problems and disorders such as diarrhoea[11], cancer[12], small pox[13], muscle catches, sprains[14] and bone fracture[15]. Recently, Sainuddin reported that ethanolic extract of *P. scandens* is affective in wound healing[16]. Even though the whole plant possesses so many medicinal properties, extensive research on phytochemical and pharmacological investigations of this plant have never been carried out. Therefore, the present study has been designed to analyze the *in vitro* antioxidant and antipyretic properties of leaf, stem and root extracts of *P. scandens*.

2. Materials and methods

2.1. Collection and identification of plant material

Fresh plants were collected during the month of October 2009 from Kottayam district of Kerala, India. The taxonomic identity of the plant was confirmed from the Botanical survey of India, Southern Circle, Coimbatore, Tamil Nadu and the voucher specimen (No: BU2065) was deposited in the Botany department herbarium, Bharathiar University, Coimbatore, India. The fresh plant materials were washed under running tap water to remove the surface pollutants and the different parts such as leaf, stem and root were separated mechanically. The separated plant parts were air dried under shade separately. Then they were separately homogenized into fine powder using mixer and used for further studies.

2.2. Chemicals and reagents

2, 2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2,2′-azinobis (3-ethyl- benzothiozoline) -6-sulfonic acid disodium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), BHT, rutin, gallic acid, ferrous chloride, ferric chloride, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra-acetic acid (EDTA) disodium salt, *N*-(1-naphthyl) ethylene diamine dihydrochloride, sodium nitroprusside, riboflavin *etc.* were purchased from Himedia, Mumbai, India. All chemicals and solvents used were of analytical grade.

2.3. Extraction of plant material

The powdered plant materials such as leaf, stem and root were packed in small thimbles separately and extracted successively with different solvents such as petroleum ether, benzene, chloroform, ethyl acetate, acetone, methanol and ethanol in the increasing order of polarity using soxhlet apparatus. Each time before extracting with the next solvent, the thimble was air dried. Finally, the material was macerated using hot water with constant stirring for 24 h and the water extract was filtered. The different solvent extracts were concentrated by rotary vacuum evaporator and then air dried.

2.4. Qualitative phytochemical screening

The leaf, stem and root extracts of *P. scandens* were analyzed for the presence of major phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, tannins, flavonoids, glycosides, flavanol glycosides, cardiac glycosides, phytosterols, fixed oils & fats, and gums & mucilages according to standard methods[17].

2.5. Quantification assays

2.5.1. Quantification of total phenolics and tannins

The total phenolics of the different plant extracts were determined according to the method described by Makkar[18]. In this method 50 μL of different plant extracts were taken into a series of test tubes and made up to 1 mL with distilled water. 1 mL of distilled water taken in a test tube served as the blank. Then, 500 μL of Folin-Ciocalteau Phenol reagent (1 N) was added to all the test tubes including the blank. After 5 minutes, 2.5 mL of sodium carbonate solution

(20%) was added to all the test tubes. All the test tubes were vortexed well to mix the contents and incubated in dark for 40 minutes. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation the absorbance was read at 725 nm against the reagent blank. Gallic acid standard was also prepared and the results were expressed as gallic acid equivalents (GAE). The analyses were performed in triplicates.

The total phenolics contain both tannin and non tannin phenolics. The amount of total tannins was calculated by subtracting the total non tannin phenolics from total phenolics[18]. For the determination of total non tannin phenolics, 500 μL of each plant samples were incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 μL of distilled water taken in a 2 mL eppendorf tube for 4 hours at 4 ℃. After incubation the eppendorf tubes were centrifuged at 4 000 rpm for 10 minutes at room temperature. The supernatant contains only the non tannin phenolics since the tannins would have been precipitated along with PVPP. The supernatant was collected and the non tannin phenolics were determined by the same method described for the quantification of total phenolics. The analyses were also performed in triplicates and the results were expressed in Gallic acid equivalents. From these two results, the tannin content of the plant samples were calculated as follows: Tannins (%) = Total phenolics (%)-Non tannin phenolics (%).

2.5.2. Quantification of total flavonoids

The flavonoid contents of all the extracts were quantified according to the method described by Zhishen *et al*[19]. About 500 μL of all the plant extracts were taken in different test tubes and 2 mL of distilled water was added to each of the test tube. A test tube containing 2.5 mL of distilled water served as blank. Then, 150 μL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation, 150 μL of 10% $AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2 mL of 4% NaOH was added to all the test tubes which were made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. Rutin was used as the standard for the quantification of flavonoids. All the experiments were done in triplicates and the results were expressed in rutin equivalents (RE).

2.6. In vitro antioxidant assays

2.6.1. $ABTS^{\bullet+}$ radical scavenging activity

The total antioxidant activity of the samples was measured by ABTS radical cation decolourization assay according to the method of Re *et al*[20]. $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1 : 89 v/v) and equilibrated at 30℃ to give an absorbance of 0.70±0.02 at 734 nm. The stock solution of the sample extracts were diluted such that after introduction of 10 μL aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 μL of sample or Trolox (final concentration 0-15 μM) in ethanol, absorbance was measured at 30 ℃ exactly 30 minutes after the initial mixing. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated against the blank (ethanol) absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity is defined as the concentration of Trolox having equivalent antioxidant activity expressed as μM/ g sample extracts.

2.6.2. Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al*[21]. An aliquot of 100 μL of sample or ascorbic acid in 1 mM dimethyl sulphoxide (standard) or distilled water (blank) was added with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a test tube. The test tubes were covered with foil and incubated in a water bath at 95 ℃ for 90 minutes. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against the reagent blank. The results reported are mean values expressed as milligrams of ascorbic acid equivalents per gram extract.

2.6.3. DPPH radical scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Blios[22]. Sample extracts at various concentrations were taken and the volume was adjusted to 100 μL with methanol. About 5 mL of a 0.1 mM methanolic solution of DPPH was added to the aliquots of samples and standards (BHT and Rutin) and shaken vigorously. Negative control was prepared by adding 100 μL of methanol in 5 mL of 0.1 mM methanolic solution DPPH. The tubes were allowed to stand for 20 minutes at 27 ℃. The absorbance of the sample was measured at 517 nm against the blank (methanol). Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of $DPPH^{\bullet}$ concentration.

2.6.4. Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of different extracts of samples were estimated according to the procedure described by Pulido *et al*[23]. FRAP reagent (900 μL), prepared freshly and incubated at 37℃, was mixed with 90 μL of distilled water and 30 μL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37 ℃ for 30 minutes in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent was prepared by mixing 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer (pH-3.6). At the end of incubation, the absorbance readings were taken immediately at 593 nm against the reagent blank, using a

spectrophotometer. Methanolic solutions of known Fe (Ⅱ) concentration, ranging from 100 to 2 000 μM, ($FeSO_4 \cdot 7H_2O$) were used for the preparation of the calibration curve. The parameter Equivalent Concentration was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4 \cdot 7H_2O$. Equivalent concentration was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mM concentration of Fe (Ⅱ) solution.

2.6.5. Metal chelating activity

The chelating of ferrous ions by various extracts of *P. scandens* was estimated by the method of Dinis *et al*[24]. Initially, about 100 μL the extract sample was added to 50 μL solution of 2 mM $FeCl_2$. The reaction was initiated by the addition of 200 μL of 5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). The metal chelating agent EDTA was used as the reference standard and results were expressed as mg EDTA equivalents/g extract.

2.6.6. Superoxide radical scavenging activity

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system[25]. Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH-7.6), 20 μg riboflavin, 12 mM EDTA, 0.1 mg NBT and 100 μL sample solution or standards (BHT and rutin). Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination, the absorbance was measured at 590 nm against the reagent blank (reaction mixture without plant sample). Identical tubes with reaction mixture kept in the dark served as negative control. The scavenging activity on superoxide anion generation was calculated as: Scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$, where, A_0 is the absorbance of the control, and A_1 is the absorbance of the sample extract/standard.

2.6.7. Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*[26]. A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (0.2 M, pH-7.4) and its concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity 81 M^{-1}/cm. The plant extracts (100 μg/mL), BHT and rutin (10 μg/mL) were added to 3.4 mL of phosphate buffer together with hydrogen peroxide solution (0.6 mL). The identical reaction mixture without the sample was taken as negative control. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against the blank (phosphate buffer). The scavenging activity (%) was calculated as: Scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$, where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

2.6.8. Nitric oxide scavenging activity

The procedure is based on the method of Sreejayan and Rao, where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent[27]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline (0.2 M, pH-7.4) was mixed with 100 μL sample solution of various extracts or BHT and rutin (standard) and incubated at room temperature for 150 minutes. The same reaction mixture without the sample was used as the negative control. After the incubation period, 0.5 mL of Griess reagent [1% sulfanilamide, 2% H_3PO_4 and 0.1% *N*-(1-naphthyl) ethylene diamine dihydrochloride] was added. The absorbance of the chromophore formed was read at 546 nm against the blank (phosphate buffer). The scavenging activity (%) was calculated as: Scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$, where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

2.7. In vivo studies

Wistar albino rats of 200-250 g were used and the *in vivo* studies were carried out at KMCH College of pharmacy, Coimbatore, Tamil Nadu.

2.7.1. Ethics

The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment (No. KMCRET/M.Sc./1/2009-10).

2.7.2. Acute toxicity

Acute oral toxicity study was performed according to the acute toxic class method[28]. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 1 000 mg/kg body weight.

2.7.3. Antipyretic activity

Antipyretic activity was measured by slightly modifying the method described by Adams *et al*[29]. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Pyrexia was induced by subcutaneously injecting 15% w/v brewer's yeast suspension (10 mL/kg) into the animal's dorsum region. 18 h after the injection, the rectal temperature of each rat was measured using a digital thermometer (BD, model No: 524928). Only rats that showed an increase in temperature at 0.7 ℃ were used for experiments. The rats were divided into four groups of six animals each. The first group was treated as control whereas the second group as standard. Group III and IV were named as dose 1 and dose 2 respectively. Paracetamol (150 mg/kg) was administered orally to the rats

of the second group. The methanol extract of root in doses of 200 and 400 mg/kg was also administered orally to the rats of third and fourth group respectively. The rectal temperatures of all the rats were measured at 1, 2, 3, 4 and 5 h after drug administration and were compared with the control and standard groups.

2.8. Statistical analysis

The results were expressed as Mean±SD. The results were statistically analyzed using one way ANOVA followed by Duncan's test for antioxidant studies and by Dunnet's *t*-test for antipyretic study. Mean values were considered statistically significant when $P < 0.05$.

3. Results

3.1. Extract recovery percent

The maximum yield was obtained in hot water compared to other solvents used. In case of leaf, maximum yield was obtained from the hot water and methanol extracts which were 8.5 and 7.7 g/100 g of dried powder respectively. The yield percentage in lower polar solvents was comparatively very low for all the parts. The higher recovery percent in hot water and methanol reveals that the plant may contain more polar compounds than non polar compounds.

3.2. Qualitative phytochemical screening

The phytochemical screening on *P. scandens* revealed the presence of primary metabolites such as carbohydrates, proteins, amino acids, fixed oils and fats in appreciable amount in all the parts. The secondary metabolites such as alkaloids, saponins, phenolic compounds, tannins, flavonoids and glycosides were found to be variously distributed in different parts of the plant (Table 1).

Table 1
Phytochemical screening of *P. scandens*.

Phytochemicals	Leaf	Stem	Root
Carbohydrates	+++	++	++
Proteins	++	+++	++
Amino acids	++	+	++
Alkaloids	++	++	+++
Saponins	+++	++	+
Phenolic compounds	+	++	+++
Tannins	+	+++	++
Flavonoids	++	+++	+++
Glycosides	++	+	+
Flavonol glycosides	–	–	–
Cardiac glycosides	+	+	+
Phytosterols	+	–	–
Fixed oils and fats	+++	++	++
Gums and mucilages	–	–	–

(+): Presence of chemical compound, (–): Absence of chemical compound.
(+) < (++) < (+++): Based on the intensity of characteristic colour.

3.3. Quantification of total phenolics, tannins and flavonoids

The total phenolics, tannin and flavonoid contents of leaf, stem and root of *P. scandens* are expressed in Table 2. The total phenolics were found to be higher in ethanol extract of stem (238.5 mg GAE/g extract). Among the different parts analyzed, stem extracts showed better phenolic content as compared to that of leaf and root. The total phenolics of the different parts follows the trend, stem > root > leaf. The acetone, methanol and ethanol extracts of all the parts used were found to have appreciable amount of total phenolics ranging from 111.1 to 238.5 mg GAE/g extract. However, the absolute ethanol was found to be more efficient solvent for extracting the phenolics from leaf, stem and root of *P. scandens*.

The total tannins were found to be higher in ethanol and methanol extracts of stem, which were 156.0 and 142.4 mg GAE/g extract respectively. The solvents such as ethanol and methanol showed better extend of extraction of tannins from leaf, stem and root of *P. scandens*. However, the amount of tannins in ethanol extracts of leaf and root were found to be 109.9 and 132.2 mg GAE/ g extract, respectively. Through the comparison of total phenolics and total tannins, it was found that the proportion of free phenolics was higher in ethanol and methanol extracts of root.

Among the different parts used, root was found to have appreciable flavonoid content in extracts such as ethyl acetate, acetone, methanol and ethanol which were 99.4, 124.8, 94.5 and 122.3 mg RE/g extract, respectively. From these data it is clear that the acetone fraction of root has higher flavonoid content as compared to other fractions of root. It was also noted that the acetone can be considered as an efficient solvent for better extraction of flavonoids from *P. scandens*. The flavonoid content of all the parts of *P. scandens* was found to be in the decreasing order of root > stem > leaf in most of the extracts.

3.4. In vitro antioxidant assays

3.4.1. $ABTS^{\bullet+}$ radical scavenging activity

The results of $ABTS^{\bullet+}$ cation radical scavenging activities of different extracts of leaf, stem and root of *P. scandens* are presented in Table 3. BHT and rutin showed 12 454.2 and 11 096.8 μM TE/g extract, respectively. The methanol and ethanol extracts of root showed higher radical scavenging activities (8 221.5 and 7 026.7 μM TE/g extract, respectively) as compared to that of other solvent extracts. However, the root was shown to have more total antioxidant activity in most of its solvent extracts. The leaf extracts of *P. scandens* made known to have total antioxidant activity ranging from 1 255.5 to 3 229.9 μM TE/g extract. The higher scavenging activity of stem was observed in its ethanol fraction and was 5 386.5 μM TE/g extract. The leaf, stem and root fractions obtained by solvents such as petroleum ether, benzene, chloroform and ethyl acetate did not give any appreciable antioxidant activity.

3.4.2. Phosphomolybdenum assay

The total antioxidant capacity of different solvent extracts of leaf, stem and root of *P. scandens* were analyzed and shown in Table 3. BHT and rutin showed 478.5 and 452.7 mg

AAE/g extract, respectively. Of the different parts used, stem showed superior activity in most of its solvents compared to the extracts of leaf and root. The better antioxidant capacity was shown by acetone extract of leaf (430.8 mg AAE/g extract) and was comparable to that of BHT and rutin. The total antioxidant capacity of the root extracts were found to be ranging from 167.3 to 325.4 mg AAE/g extract. Among the different solvents used the acetone extracts of all the parts showed better antioxidant capacity as compared to other solvent extracts.

3.4.3. DPPH radical scavenging activity

The DPPH radical scavenging activities of different extracts of leaf, stem and root of *P. scandens* are shown in Figure 1. DPPH is a stable free radical which is commonly used for assessing antioxidant activity in plant samples. Usually, the results of DPPH assay were expressed in IC_{50} values. The lower value of IC_{50} indicates a higher antioxidant activity. Among all the parts studied, the ethanol extracts of stem and root showed better $DPPH^{\cdot}$ radical scavenging activities compared to other solvent extracts. The IC_{50} of ethanol extract of root was found to be 0.284 mg/mL whereas that of leaf and stem was 0.331 and 0.292 mg/mL, respectively. The IC_{50} of standard antioxidants were found to be much far from that of plant extracts, revealing that the plant has less free radical scavenging activity compared to vitamin E, rutin, BHT and BHA.

3.4.4. FRAP assay

Antioxidant potential of leaf, stem and root of *P. scandens* were estimated from their ability to reduce TPTZ– Fe (Ⅲ) complex to TPTZ– Fe (Ⅱ) and are given in Table 4. The lower value in the table represents good antioxidant activity. Among the different solvent extracts estimated, methanol and ethanol extracts showed comparable ferric reducing antioxidant activity. The methanol extract of root has been shown to possess higher ferric reducing antioxidant activity and was 506.5 mM Fe (Ⅱ)/mg extract whereas in case of stem, the better activity was shown by ethanol extract. Among all the extracts of different parts, the ferric reducing power of root extracts was higher than the corresponding extract of leaf and stem. The ferric reducing power of methanol extracts were 548.7 and 670.5 mM Fe (Ⅱ)/mg extract for leaf and stem, respectively. The ethanol extracts of all the parts showed better activity compared to other solvent extracts such as ethyl acetate, acetone chloroform *etc*. and were 609.3, 542.0 and 514.4 mM Fe (Ⅱ)/mg extract for leaf, stem and root respectively.

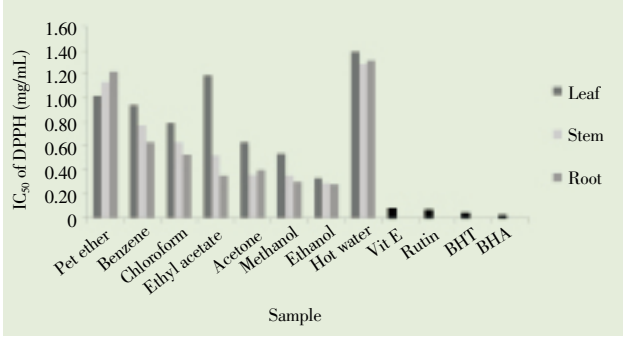

Figure 1. DPPH radical scavenging activity of *P. scandens*.

3.4.5. Metal chelating activity

Among the different extracts used for the parts, hot water and ethanol extracts showed better metal chelating ability in all the parts compared to other solvent extracts (Table 4). The metal chelating capacity of hot water extracts of leaf, stem and root were found to be 1 073.3, 986.4 and 1 011.6 mg EDTA equivalents/g extract respectively. The ethanol and ethyl acetate extracts also showed better metal chelating capacity ranging from 692.4 to 913.8 mg EDTA equivalents/g extract.

3.4.6. Superoxide radical scavenging activity

The superoxide anion scavenging activities of leaf, stem and root of *P. scandens* were determined at 100 μg/mL concentration and are shown in Figure 2. The extracts were found to be an efficient scavenger of superoxide radical generated in riboflavin–NBT–light system *in vitro*. The ethyl acetate and ethanol fractions of all the parts showed higher activity compared to other solvent extracts. The scavenging activity of stem was found to be 69.62% for ethyl acetate and 66.92% for ethanol and was comparable to that of BHT and rutin. Among the different parts, the scavenging activity for all the samples ranged from 20.02% to 69.62%.

3.4.7. Hydrogen peroxide scavenging activity

The scavenging ability of various extracts with hydrogen peroxide is shown in Figure 3. It was also noticed that all the extracts are capable of scavenging hydrogen peroxide in an amount–dependent manner. The higher percentages of scavenging activity were found in root methanol (60.3%) and ethanol extracts (58.4%) at a concentration of 100 μg/mL. The scavenging activity of ethanol extracts of leaf and stem were found to be 52.3% and 56.0%, respectively which was also far from that of BHT and rutin.

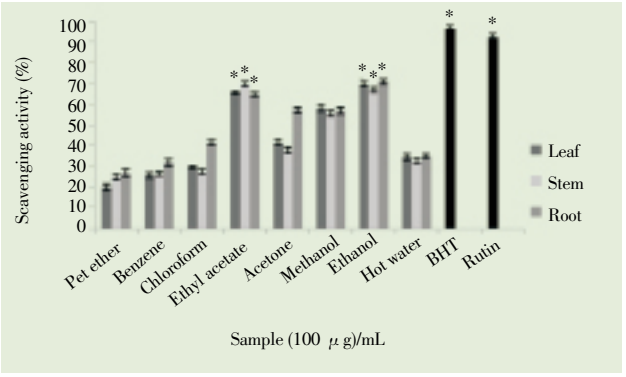

Figure 3. Scavenging activity of *P. scandens* on superoxide radical.
*: statistically significant at $P<0.05$.

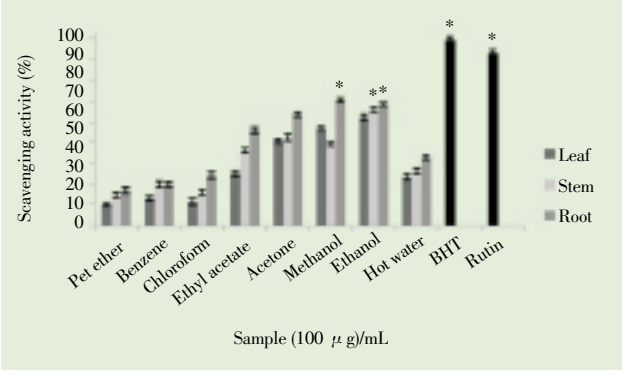

Figure 3. Scavenging activity of *P. scandens* on hydrogen peroxide
*: statistically significant at $P<0.05$.

3.4.8. Nitric oxide scavenging activity

The scavenging activities of various samples on nitric oxide were analyzed and the percentage of scavenging activities of leaf, stem and root of *P. scandens* are shown in Figure 4. At a concentration of 100 μg/mL, the greater percentages of scavenging activities were observed in acetone, methanol and ethanol extracts for all the parts analyzed. The methanol extract of root showed the greater percentage of scavenging activity (58.7%) whereas the scavenging activities of ethanol extracts of leaf, stem and root were found to be 51.8%, 52.1% and 57.3%, respectively. Among the different extracts of leaf, stem and root of *P. scandens*, the methanol extract of *P. scandens* root showed better antioxidant activity comparable to other extracts. Therefore, the methanol extract of root was used for further *in vivo* studies to reveal the pharmacological properties.

3.5. In vivo studies

3.5.1. Acute toxicity

The methanol extract of *P. scandens* root was evaluated for its acute toxicity in rats. The extract did not alter the general behavior and failed to produce any mortality even at highest dose of 1 000 mg/kg. In acute toxicity study, the extract was found to be safe at 1 000 mg/kg and reveals that this plant might be considered as a broad nontoxic one.

3.5.2. Antipyretic activity

The antipyretic activity of root extract of *P. scandens* is shown in Table 5. The results showed that the methanol extract of root at dose of 200 mg/kg caused significant lowering of the body temperature up to 5 hours following extract administration, as the normal mean temperature 38.53 ℃ at 0 h was reduced to 37.1 ℃ at 5 h. While maximum lowering of body temperature was noticed at 400 mg/kg of the root methanol extract, as the mean temperature of 39.43 ℃ was reduced to 38.41 ℃ within 3 h period in a dose dependent manner. The data revealed that the rectal temperature of 37.35 ℃ at 0 h was markedly elevated to 39.38 ℃ for control and 39.18 ℃ for paracetamol group (standard) 18 h after the subcutaneous injection of yeast suspension. The animals treated with methanol extract at 200 and 400 mg/kg doses showed a decrease in the rectal temperature by 0.19 and 0.34 ℃, respectively within 1 h. On the other hand, in 5 h the temperature was reduced by 1.5 ℃ for all the groups of animals received the extract.

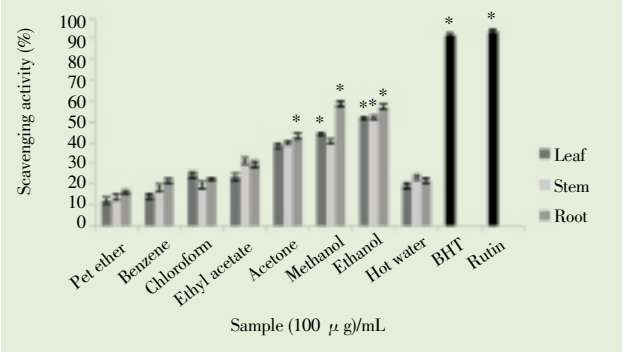

Figure 4. Scavenging activity of *P. scandens* on nitric oxide.
*: statistically significant at $P<0.05$.

4. Discussion

4.1. Qualitative phytochemical screening

The presence of carbohydrates, proteins and amino acids in all the parts of the plant reveals that it can be used as a good nutritional supplement, if it is free from antinutritional factors. It is also noted that the other phytochemical compounds detected are known to have beneficial use in pharmaceutical industries. The higher amount of alkaloids present in all the parts of the plant can make the plant to be a good source of alkaloids which can be isolated and then purified. In medicine, saponin is used in hypercholestrolaemia, hyperglycaemia and as antioxidant, anticancer, anti-inflammatory *etc*[30]. Therefore the presence of higher amount of saponins in leaves of *P. scandens* makes it a good source for both industrial and medical purposes.

Tannins were reported to exhibit antiviral, antibacterial and antitumour activities. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as a diuretic. Since all the parts of plant contain total tannin in appreciable amounts, it is clear that *P. scandens* can have the ability to cure such viral and bacterial diseases. The phenolics and flavonoids have got much attention in the day to day life due to their antimutagenic, antitumour and antioxidant activities. Therefore, the estimation and characterization of stem and root extracts of *P. scandens* for phenolics and flavonoids should be done to explore bioactive principles of such compounds.

4.2. Quantification of total phenolics, tannins and flavonoids

The higher amount of phenolics in polar solvents like acetone, methanol and ethanol could be due to higher solubility of phenolics and other aroma compounds. It has been already investigated in many plant species that the total phenolics could be significantly contribute to the antioxidant capacity of that species. More likely, cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction, which may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions[31]. Therefore, the higher amount of phenolics in all the parts of *P. scandens* can be taken as a good indication for its higher antioxidant capacity.

The greater amount of tannins in the extracts of stem of *P. scandens* can be +due to higher polymerization of existing polyphenolic compounds. Recently, it has been reported that the high molecular weight phenolics such as tannins have more ability to quench/scavenge free radicals. Therefore, the high amounts of tannins in stem and root of *P. scandens* may enhance the free radical scavenging activity of its extracts.

Flavonoids are one of the most diverse and important group of natural phenolics. These compounds possess a broad spectrum of chemical and biological activities. *In vitro* and animal studies have shown that flavonoids possess anti-inflammatory, antioxidant, antiallergenic, hepato-protective, antithrombotic, antiviral and anticarcinogenic activities[5]. In addition, flavonoids have been identified as fulfilling most of the criteria to be considered as antioxidants: the flavonoids inhibit the enzymes responsible for superoxide

Table 2

Total phenolics, tannin and flavonoid contents of *P. scandens*(mean ± SD, *n*=3).

Solvents	Phenolics (mg GAE/g extract)			Tannins (mg GAE/g extract)			Flavonoids (mg RE/g extract)		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Petroleum ether	36.2±2.6	51.5±2.1	57.4±1.8	14.1±2.3	11.8±2.4	10.7±1.9	11.3±2.1	10.4±2.3	14.8±2.4
Benzene	41.1±2.2	67.4±1.7	70.9±1.8	10.2±1.7	16.1±2.8	17.9±2.6	17.1±1.8	19.8±2.1	18.7±2.3
Chloroform	52.2±1.1	71.1±2.8	94.3±1.4	13.5±1.8	18.3±2.2	20.6±2.5	20.2±1.9	26.9±3.6	23.4±3.2
Ethyl acetate	67.5±2.0	112.2±1.9	143.7±1.2	25.2±1.2	45.3±2.3	46.0±2.9	64.1±2.8	69.2±2.3	99.4±1.7*
Acetone	111.1±2.7	185.6±2.4*	161.1±1.9*	40.8 ±2.5	112.5±2.3*	73.6±2.2	93.6±2.5*	98.6±1.6*	124.8±2.3*
Methanol	156.9±2.7*	204.6±2.1*	197.5±2.3*	83.4 ±2.9	142.4±2.7*	105.5±1.8	59.3±2.2	68.0±2.6	94.5±1.9*
Ethanol	202.9±2.9*	238.5±1.9*	231.9±2.5*	109.9 ±3.6	156.0±2.5*	132.2±3.0*	84.4±2.1*	77.9±2.4	122.3±2.7*
Hot water	83.5±1.7	67.4±2.5	96.7±2.1	58.6±2.3	26.3±1.9	62.9±2.3	31.3±1.8	41.4±2.1	37.6±1.8

* Statistically significant at $P<0.05$. GAE: Gallic acid equivalents; RE: Rutin equivalents.

Table 3

$ABTS^{\bullet+}$ radical scavenging activity and Phosphomolybdenum assay of *P. scandens*(mean ± SD, *n*=3).

Solvents	$ABTS^{\bullet+}$ radical scavenging (μ M TE/ g extract)			Phosphomolybdenum assay (mg AAE/ g extract)		
	Leaf	Stem	Root	Leaf	Stem	Root
Petroleum ether	1 493.4±149.7	1 488.4±122.5	1 093.5±124.1	211.5±19.0	188.5±11.7	175.2±12.2
Benzene	2 841.2±115.7	3 057.7±165.4	3 078.0±202.9	222.8±17.3	248.1±20.1	201.9±19.1
Chloroform	2 500.9±193.1	3 452.6±137.0	4 606.8±146.7	237.5±15.7	271.2±14.9	219.2±17.2
Ethyl acetate	2 156.6±161.6	3 908.2±116.4	4 627.1±144.7	244.9±16.2	276.9±10.9	223.1±13.5
Acetone	1 255.5±137.3	3 169.1±158.2	3 624.7±170.0	430.8±14.4*	357.7±12.7*	290.8±17.2
Methanol	2 257.9±138.0	4 181.6±119.8	8 221.5±148.4*	219.2±17.2	226.9±14.5	281.2±22.2
Ethanol	2 956.5±107.9	5 386.5±104.0	7 026.7±113.9*	274.8±13.1	305.8±14.0*	325.4±10.5*
Hot water	3 229.9±146.8	2 885.6±168.7	2 197.1±189.9	196.2±20.8	192.3±13.5	167.3±20.4

*: Statistically significant at $P<0.05$. TE: Trolox equivalents; AAE: Ascorbic acid equivalents.

Table 4

FRAP assay and Metal chelating activity of *P. scandens*(mean ± SD, *n*=3).

Solvents	FRAP (mM Fe (II)/mg extract)			Metal chelating (mg EDTA Equivalents/g extract)		
	Leaf	Stem	Root	Leaf	Stem	Root
Petroleum ether	1 197.3 ± 23.3	1 264.0 ± 21.9	1 160.3 ± 23.8	159.8 ± 11.2	126.9 ± 9.7	141.6 ± 12.1
Benzene	996.7 ± 16.7	918.0 ± 20.4	1 028.6 ± 18.3	207.4 ± 9.4	197.1 ± 11.3	208.4 ± 9.2
Chloroform	944.1 ± 14.8	887.3 ± 23.8	862.1 ± 17.4	268.3 ± 8.9	221.1 ± 10.6	313.9 ± 9.6
Ethyl acetate	760.4 ± 9.4	732.1 ± 15.4	740.7 ± 14.5	726.1 ± 7.6	692.4 ± 7.3	736.3 ± 10.4
Acetone	626.9 ± 17.5	822.7 ± 16.0	778.9 ± 20.6	378.3 ± 10.1	325.5 ± 8.5	412.8 ± 8.5
Methanol	548.7 ± 14.1	670.5 ± 22.6	506.5 ± 16.4*	551.2 ± 12.3	509.6 ± 7.9	624.7 ± 8.2
Ethanol	609.3 ± 14.2	542.0 ± 12.8*	514.4 ± 16.9*	913.8 ± 8.1*	814.5 ± 8.7	796.6 ± 7.6
Hot water	930.5 ± 17.4	828.2 ± 13.7	805.3 ± 10.2	1 073.3 ± 9.5*	986.4 ± 10.5*	1 011.6 ± 9.1*

*: Statistically significant at $P<0.05$.

Table 5

Effect of *P. scandens* root methanol extract (PSRM) on Brewer′s yeast-induced pyrexia in rats (mean ± SEM, *n*=6).

Treatment	Dose (mg/kg)	Rectal temperature (℃)						
		-18 h	0 h	1 h	2 h	3 h	4 h	5 h
Control	-	37.35±0.12	39.38±0.18	39.47±0.15	39.41±0.19	39.13±0.13	39.20±0.14	39.05±0.18
Paracetamol	150	37.28±0.31	39.18±0.24	38.85±0.21*	38.68±0.22*	38.26±0.26***	38.06±0.24**	37.68±0.25***
PSRM	200	36.18±0.25**	38.53±0.29**	38.34±0.32**	38.18±0.26***	37.21±0.21***	37.45±0.29***	37.10±0.31***
PSRM	400	37.95±0.33	39.43±0.30	39.09±0.28	38.75±0.31*	38.27±0.27**	38.14±0.32**	38.08±0.30**

Significantly different at * $P<0.05$, ** $P<0.01$, *** $P<0.001$ when compared to control.

radical production, the low redox potentials of flavonoids thermodynamically allow them to reduce highly oxidizing free radicals and a number flavonoids chelate trace metals[32]. Therefore, the quantification of flavonoid content of plant parts is important, since it can be correlated with the radical scavenging activity of the plant. In view of the fact that since *P. scandens* possess good flavonoid content in all the parts, it could be assumed that it can have a higher free radical scavenging activity.

4.3. $ABTS^{\bullet+}$ radical scavenging activity

The ABTS radical is soluble in both aqueous and organic solvents, is not affected by ionic strength and can be used to measure the antioxidant capacity of hydrophilic and lipophilic compounds in test samples[33]. Total antioxidant activity (TAA) of samples having higher phenolic content seems to be efficient for functioning as potential nutraceuticals or antioxidants when they are ingested along with nutrients. The radical is suitable for evaluating antioxidant capacity of phenolics due to their comparatively lower redox potentials(0.68V). Many phenolic compounds can thus react with the ABTS radical because of this thermodynamic property[34]. The total antioxidant activity shown by the extracts of *P. scandens* points out that it may due to the higher amount phenolics and tannins which seemed to act as good radical scavengers.

4.4. Phosphomolybdenum assay

Phosphomolybdenum assay is successfully used to quantify vitamin E in seeds, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts[20]. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. Thus, the total antioxidant capacity observed for the extracts of *P. scandens* can be correlated with its free radical scavenging activity equivalent to that of natural antioxidant ascorbic acid.

4.5. DPPH radical scavenging activity

The DPPH assay is commonly used because it is technically simple and gives accurate and repeatable results. The assay is valid to quantify samples with hydrophilic or lipophilic antioxidants[8]. Even though the DPPH radical scavenging activities of different extracts of the plant were seemed to be an average, it is not confirmed that the plant has no potential radical scavenging activities. The antiradical scavenging activity of different extracts of *P. scandens* would be related the nature of phenolics, thus contributing to their electron transfer/ hydrogen donating ability[22]. It is also found that the antioxidants that react quickly with peroxyl radicals may react slowly or may be inert to the DPPH radical[35]. Steric accessibility is a major determinant of the reaction mechanisms, hence small molecules have higher apparent antioxidant capacity due to their better access to the DPPH radical site[36]. Thus it can be assumed that *P. scandens* may contain antioxidants of higher molecular value which may not be able to counteract with DPPH. Therefore, further antioxidant assays should be carried out to assess the quantity of antioxidants in *P. scandens*.

4.6. FRAP assay

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant. The ferric reducing antioxidant power assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. Siddhuraju *et al*. has reported that the reducing power of bioactive compounds, mainly low and high molecular phenolics, was associated with antioxidant activity, specifically scavenging of free radicals[37]. It has been also proved that the potential antioxidants through *in vitro* ferric-reducing antioxidant power assay increased the total antioxidant capacity of blood plasma[38]. Thus the ferric reducing power the different extracts of *P. scandens* reveals that there are compounds in the methanolic and ethanolic extracts which have high affinity to the ferrous ions and thereby quench/ scavenge them through redox reactions.

4.7. Metal chelating activity

Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation[39]. Through epidemiological studies, it was reported that phenolic compounds have been shown to act as natural antioxidants by helping to neutralize free radicals and as metal chelating agents[40]. Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion. Hence the data obtained for *P. scandens* reveals that some of the extracts demonstrate an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron binding capacity that will prevent the free radical generation through Fenton reaction.

4.8. Superoxide radical scavenging activity

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced *in vivo* and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, *eg*., the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals[41]. Numerous biological reactions generate superoxide radical which is a highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potent precursors of highly reactive species such as hydroxyl radical and thus the study of scavenging of this radical is important[42]. Since *P. scandens* showed appreciable percentage of scavenging activity for hydrogen peroxide, it can be used against unfavourable effects caused in the body by hydrogen

peroxide.

4.9. Hydrogen peroxide scavenging activity

Hydrogen peroxide itself is not very reactive but sometimes it is toxic to cell because it may give rise to hydroxyl and peroxyl radicals in the cells through Fenton reaction[41]. Therefore, removing of H_2O_2 is very important for antioxidant defence in cell or food systems. Dietary polyphenols have also been shown to protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide, especially compounds with the orthodihydroxy phenolic structure, quercetin, catechin, gallic acid ester and caffeic acid ester[43-50]. Therefore, the methanol and ethanol extracts of *P. scandens* can be used as a potent hydrogen peroxide scavenger in body systems.

4.10. Nitric oxide scavenging activity

Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO_2, N_2O_4, N_3O_4, NO_3^- and NO_2^+ are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric acid is also implicated for inflammation, cancer, and other pathological conditions[51]. Since the ethanol extracts of *P. scandens* showed good activity it is clear that it can be used for scavenging reactive nitrogen species in human body.

4.11. Antipyretic activity

The results showed that the methanol extract of root of *P. scandens* possesses a significant antipyretic effect in maintaining normal body temperature and reducing yeast-induced elevated body temperature in rats in a dose dependent manner and its effect is comparable to that of the standard antipyretic drug paracetamol. Furthermore, the significant reduction of yeast provoked elevated temperature of the tested animals by the extract at 200 mg/kg dose and 400 mg/kg of fractions appears to be due to the action of ursolic acid, β-sitosterol and its glucoside alone or in combination. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus[52]. Therefore, the antipyretic activity of methanolic extract of *P. scandens* is probably by inhibition of prostaglandin synthesis in hypothalamus. The antipyretic activities of methanolic extract can also be due to the presence of alkaloids, sterols and flavonoids. It was evident from the study that the observed antipyretic effects of the extract were similar in both magnitude and time course. However, to know the exact mechanism of action of *P. scandens* root extract further study with purified fractions is warranted.

From our investigation it can be concluded that *P. scandens* possess strong antioxidant and antipyretic activity which may be influenced by the phenolic and flavonoid contents. Further, detailed exploration, chemical studies and screening for medicinal properties will provide cost effective and reliable source of medicine for the welfare of humanity.

Conflict of interest statement

We declare that we have no conflict of interest.

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